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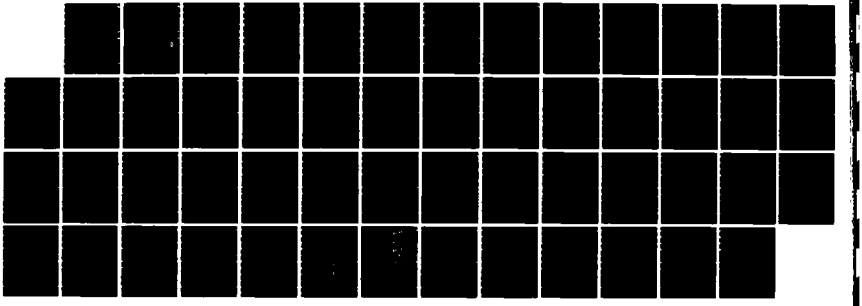

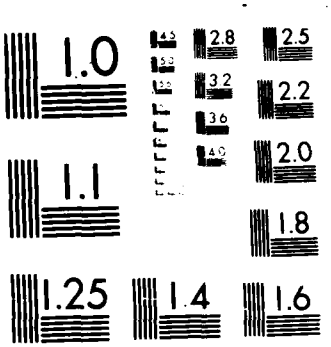

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# PHARMACOLOGICAL SPARING OF PROTEIN AND GLUCOSE IN BURN INJURY AND/OR SEPSIS

ANNUAL SUMMARY REPORT

Robert R. Wolfe, Ph.D.

James H. F. Shaw, M.D.

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## SUMMARY

We have assessed the role of glucagon and sympathetic nervous system (SNS) activity on glucose and palmitate kinetics and oxidation in the conscious dog infused with live E. coli bacteria by means of the simultaneous primed constant infusion of 1,2,$^{13}$C-palmitate and U-$^{14}$C-glucose. The basal rate of glucose production in septic dogs and controls was similar. However, when the glucagon concentration was selectively decreased in the septic animals by the appropriate infusion of somatostatin (S), insulin (I), and glucagon (G), the rate of glucose production decreased significantly, whereas in control animals, S + I + G infusion had no effect on glucose kinetics. When α and β blockade was added to the infusion, the rate of glucose production decreased further and hypoglycemia developed in the septic dogs, whereas in the controls both glucose production and concentration increased. The basal rate of appearance of palmitate was increased in the septic dogs ($P < 0.001$). S + I + G had no effect on palmitate appearance in either group, and sympathetic blockade caused a significant decrease in palmitate appearance in both groups of dogs. The rates of oxidation of both glucose and palmitate was related directly to their availability in plasma. Thus, in sepsis glucagon and SNS activity play important roles in the mobilization of glucose and palmitate into plasma, and therefore in the overall state of energy metabolism.

The effect of gram-negative sepsis on the kinetics and oxidation of very-low-density-lipoprotein (VLDL) fatty acids was also assessed in conscious dogs in the normal state and 24 h after infusion of live Escherichia coli. VLDL, labeled with 2-$^{3}$-H-glycerol and 1-$^{14}$C-palmitic acid, was used to trace VLDL kinetics and oxidation, and 1-$^{13}$C-palmitic acid bound to albumin was infused simultaneously in order to quantify kinetics and oxidation of free fatty acid (FFA) into plasma. Sepsis caused a significant increase in the plasma triglyceride (TG) concentration and a fivefold increase in the rate of VLDL production (RaVLDL). The increase in RaVLDL was associated with a significant increase in plasma FFA flux (RaFFA), but the ratio of RaVLDL/RaFFA increased significantly in sepsis. In the control dogs, the direct oxidation of VLDL-fatty acids was not an important contributor to their overall energy metabolism, but in dogs with sepsis, 17% of the total rate of $CO_2$ production could be accounted for by VLDL-fatty acid oxidation. When glucose was infused into dogs with insulin and glucagon levels clamped at basal levels (by means of infusion of somatostatin and replacement of the hormones), RaVLDL increased significantly in the

control dogs, but it did not increase further in dogs with sepsis. We conclude that the increase in TG concentration in fasting dogs with gram-negative sepsis is the result of an increase in VLDL production, and that the fatty acids in VLDL can serve as an important source of energy in sepsis.

When taken together, these studies lead us to conclude that the lowering of glucagon concentration should have an important effect in reducing gluconeogenesis, and thus protein wasting. Manipulation of the sympathetic nervous system to enhance the mobilization of lipid, on the other hand, does not seem likely to be fruitful since there are already ample amounts of fatty acids available as energy substrates. Future studies will therefore focus entirely on minimizing protein breakdown directly and via suppression of gluconeogenesis, and adrenergic blockade will not be pursued.

## FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals", prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

## INTRODUCTION

Glucose metabolism in sepsis has been studied extensively but the research has yielded contradictory results. In experimental septic and endotoxemic models, animals generally die in a state of profound hypoglycemia (1), and life can be prolonged by glucose infusion (2). Studies in vitro have indicated that hypoglycemia in septic or endotoxic shock is caused at least partly by the impairment of hepatic glucose production (31, 13). Isolated hepatocytes from endotoxin-treated donors have been shown to have impaired rates of gluconeogenesis (1). The results of enzyme studies are consistent with these findings. For example, Berry, et al (4), have shown that endotoxin prevents the induction of phosphoenolpyruvate-carboxykinase (PEP-CK). Our own studies have shown that the gluconeogenic capacity is depressed in isolated perfused rat livers taken from guinea pigs infused with live bacteria (1).

In contrast, in kinetic experiments, performed in vivo, a suppression of glucose production in sepsis has generally not been documented. Patients with sepsis have been found to have rates of glucose production that are either higher than or similar to control levels (5). In vivo animal studies have similarly found glucose production to be either elevated or in the normal range of sepsis or endotoxemia, except in the agonal phase (1, 6).

Studies in vivo of the factors that regulate lipid metabolism in sepsis have also yielded conflicting results. Plasma free fatty acid levels have been reported to be depressed (7, 8), in the normal range (9, 10) or elevated (13) in a variety of septic states. Hormonal and sympathetic nervous system control of plasma FFA kinetics in sepsis have not been characterized in vivo, but fat cells obtained from endotoxin-treated animals and cells challenged with endotoxin in vitro have been shown to have normal or increased lypolytic activity (14, 15).

The concentrations of triglycerides (TG) in plasma are elevated in gram-negative sepsis in humans (9) and in various animal models (e.g., 16). This could be the result of an increased rate of production and release of very-low-density-lipoproteins (VLDL), or of a decreased ability to clear TG from plasma, or a combination of both. The plasma concentration alone does not permit a choice to be made among these possible explanations, because the flux of VLDL is not directly related to the plasma concentration in a variety of pathological states (2). The observation that TG metabolism is altered in sepsis is important because of the meaning it may have in energy metabolism

and nutrition, and also because it could explain the infiltration of the liver by fat that occurs in gram-negative sepsis in conjunction with indicators of hepatic failure (17). TG kinetics have not yet been determined in gram-negative sepsis, however, either in an animal model or in human patients.

This study was performed in part to test the hypothesis that sepsis exerts a direct inhibitory effect on the gluconeogenic capacity of the liver, but that this effect can be neutralized in vivo by increased glucagon and sympathetic nervous system activity. We also evaluated the role of hyperglucagonemia and sympathetic activity in sepsis in the regulation of plasma palmitate kinetics as an indicator of plasma free fatty acid metabolism. In addition, we tested the hypothesis that the rates of oxidation of plasma glucose and palmitate in sepsis are primarily functions of their rates of appearance in plasma, and that alteration of either the glucagon level or sympathetic activity has little direct effect on the ability of the host to oxidize these substrates.

To determine the effect of sepsis on the kinetics of a substrate, the response in the basal state should be evaluated, and then the response of the rate of flux of the substrate that occurs with a known stimulus or inhibitor should be determined. Such an approach often yields insight into the underlying reason for the observed alteration in basal kinetics. In the case of VLDL kinetics, evaluation of the response to an intravenous infusion of glucose is a convenient test of practical importance. High carbohydrate intake is known to result in hypertriglyceridemia (18), and glucose infusion is known to alter endogenous triglyceride metabolism, as evidenced by the development of fatty infiltration of the liver with prolonged infusion (19). Also, glucose infusion is the most common form of caloric support for patients with sepsis. Another goal of this experiment, therefore, was to determine the effect of sepsis on VLDL kinetics in the basal state and during an intravenous infusion of glucose.

Although high carbohydrate intake is known to stimulate VLDL production, the mechanism responsible for this response is not clear. Two possibilities are that the effect is mediated via the increase in glucose availability, per se, or alternatively, via the increase in insulin that occurs in response to the hyperglycemia. Evidence from in vitro studies supports the view that insulin stimulates VLDL production directly (20, 21), but evidence from in vivo experiments is contradictory (22, 23, 24). A secondary goal of this experiment, therefore, was to distinguish the action of hyperinsulinemia from the action of hyperglycemia on the rate of production of VLDL.

A substantial impediment in distinguishing between the actions of insulin and hyperglycemia in VLDL in vivo is that both inhibit the mobilization of free fatty acids (FFA) into plasma, and the rate of delivery of FFA to the liver is probably the most important determinant of the rate of VLDL synthesis (25). Similarly, the response of VLDL kinetics in any model of sepsis should be considered in relation to the plasma FFA flux, because FFA flux has been reported to be affected differently in different septic models (26, 27). Consequently, in this experiment, plasma FFA kinetics were determined at the same time as VLDL kinetics. We used VLDL labeled with 1-$^{14}$C-palmitate to trace VLDL kinetics and albumin-bound 1-$^{13}$C-palmitate to trace plasma FFA kinetics. Thus, in addition to tracing the plasma kinetics, we could also compare the importance (in terms of energy metabolism) of the direct oxidation of fatty acids in VLDL in relation to that of the direct oxidation of plasma FFA. The VLDL was also labeled with 2-$^{3}$H-glycerol in order to better quantitate the plasma kinetics.

## METHODS

### A. EVALUATION OF THE ROLE OF GLUCAGON AND INSULIN ON GLUCOSE AND FFA KINETICS IN SEPSIS

## MATERIALS AND METHODS

All studies were performed in conscious dogs with chronic arterial and venous lines and a chronic tracheostomy. This study was approved by the Animal Use Committee of the Shriners Burns Institute and conformed with the guidelines for animal use as promulgated by the American Physiological Society and the National Institutes of Health, and was also approved by the Annual Use Committee for the U. S. Army. The turnover rates of glucose and palmitate were assessed by simultaneous, constant infusions of U-$^{14}$C-glucose (New England Nuclear, Boston, MA) and 1,2,$^{13}$C-palmitate (Merck Isotopes, Montreal, Canada). By successfully priming the bicarbonate pool with $H^{13}CO_3$ and $H^{14}CO_3$ we were able to simultaneously measure palmitate and glucose oxidation.

### (1) Dogs

We have described the septic dog model in detail elsewhere (27). Mongrel dogs weighing 15-20 kg were anesthetized with intravenous sodium pentobarbital (Seratol) and given ampicillin (500 mg). Chronic tracheostomies were established along with arterial, venous and portal cannulae. The tracheostomies were performed by removing the anterior aspects

of three tracheal cartilages and suturing the tracheal mucosa to the overlying skin. The polyethylene cannulae (PE 90) were then inserted into one external jugular vein and one carotid artery, and the cannulae advanced into the vena cava and aorta respectively. Another cannula was advanced via the splenic vein to the portal vein. All the cannulae were externalized at the back of the neck. The dogs were allowed seven to ten days to recover from the surgery. During this time they were fed regular dog chow and canned dog food. The animals were in good health during the control studies as assessed by evaluations of their temperature, heart rate, cardiac output and general appearance. After the control studies were completed, two or three days were allowed to pass before induction of sepsis.

(2) Preparation of E. coli

E. coli were obtained in the freeze dried form from American Scientific Products (Lawrence, MA) and reconstituted in trypticase (American Scientific Products), and then transferred to agar plates. One microbiological loop of E. coli was removed from this source and innoculated in 10 mL of soy broth medium in a sterile test tube and incubated overnight at 37°C. The following day the tube was centrifuged (1500 rpm for 10 minutes), and the medium was removed by pipette. Ten mL of sterile saline was then added to the E. coli pellet and 1 mL was removed in order to count the organisms. The 1 mL aliquot was serially diluted and each dilution was plated for quantitation. The rest of the solution was used for infusion.

(3) Induction of Sepsis

The dogs were fed a mixed meal in the morning on the day of induction of sepsis. Four hours later, $10^{10}$ E. coli were infused through one arterial line over a period of 30 min. Each animal received approximately 1000 mL of lactated Ringers solution over the following three-hour period. Sixty minutes after the infusion of E. coli began, most of the dogs laid on the bottom of the cage but were alert. Again, this was given over a 30-minute period. The dogs were kept warm overnight and studied the following day. Sepsis was assessed by blood culture, degree of tachycardia, the presence of pyrexia and general appearance.

EXPERIMENTAL DESIGN

We performed 14 studies in seven dogs. Each dog was studied in the control state and in the septic state. The kinetics and oxidation of plasma glucose and palmitate were assessed in the basal state (period 1), and when insulin and glucagon were

controlled by infusing somatostatin, insulin and glucagon, (period 2). Finally, with insulin and glucagon controlled, the effect of α and β sympathetic blockade on glucose and palmitate kinetics was assessed by infusing propranolol and phentolamine (period 3). We have already demonstrated that this model allows a physiological steady state to be maintained in which glucose and palmitate kinetics are in the basal range and insulin and glucagon levels are held constant (28).

Studies in Non-Septic Animals

In the control studies (n=7) U-$^{14}$C-glucose and 1,2$^{13}$C-palmitic acid were infused throughout all periods. In period 1 (90 min) only isotopes were infused; in period 2 (60 min) hormonal control was established with somatostatin, insulin and glucagon. In period 3 (60 min) the somatostatin, insulin and glucagon were continued, and in addition, adrenergic blockade was established by infusing propranolol and phentolamine. The rates of infusion of somatostatin, insulin and glucagon were designed to maintain insulin and glucagon at the normal post-absorptive levels in control dogs.

Studies in Septic Animals

In the septic dogs U-$^{14}$C-glucose and 1,2,$^{13}$C-palmitate were also infused for evaluation of glucose and palmitate kinetics and oxidation in the basal state, under hormonal control, and under hormonal control with sympathetic activity blocked. Although 6-$^{3}$H-glucose is theoretically preferable to $^{14}$C-glucose as a tracer to measure glucose kinetics because of the recycling of $^{14}$C, we have found little difference between the values obtained in dogs with the two methods. We have recently published (28) data accumulated from different experiments in which a total of 56 dogs were studied with the two tracers simultaneously and no significant difference between the two techniques was observed.

In both the control and septic groups, samples of blood and expired air were collected over the last 30 min of period 1, and throughout periods 2 and 3. Blood samples were collected at 60, 70, 80 and 90 min of period 1; 15, 30, 45 and 60 min of period 2; 15, 30, 45 and 60 min of period 3. Expired breath was collected for $VO_2$ and $VCO_2$ determination between 0-60 min in period 1, and between 45-60 min in period 2 and 3. Samples for $CO_2$ enrichment determination were drawn at times corresponding to the times at which blood samples were drawn.

The response to adrenergic blockade was not evaluated in the absence of hormonal control because the blockade has been found to produce varying effects on glucagon and insulin levels in control and septic animals without such control (29).

Isotope Tracer Infusion

We used primed constant infusions of both stable and radio-labeled isotopes to quantitate the effect of E. coli septicemia on glucose and palmitate. By employing a $^{13}C$-labeled palmitate tracer (Merck Isotopes, Montreal, Canada) while infusing U-$^{14}C$-glucose (New England Nuclear, Boston, MA) and measuring the specific activity of $^{14}CO_2$ and the enrichment of $^{13}CO_2$, we were also able to measure substrate oxidation directly.

The U-$^{14}C$-glucose was given as a primed, constant infusion, at the rate of 20 nCi/kg·min, with a prime to infusion rate ratio of 80:1. In addition, 640 nCi/kg of $NaH^{14}CO_3$ was administered at the start of the isotope infusion in order to prime the bicarbonate pool.

Plasma palmitate metabolism was traced by using 1,2,$^{13}C$-palmitic (90% MPE) acid. In order to infuse the labeled palmitic acid, it was necessary to bind it first to albumin (Cutter, Emmeryville, CA) (20). The resulting albumin-$^{13}C$-palmitic acid solution had a concentration of approximately 2 µmol/mL The actual concentration was determined by gas-chromatography analysis. In addition, the enrichment of the palmitate infusion mixture was determined by GCMS analysis in order to account for any unlabeled palmitate already bound to the albumin. The Cutter albumin solution, however, is very low in FFA concentration, so the resulting enrichment of the isotope infusion was usually about 85%. We infused this solution through a venous line at the rate of 0.764 mL/min, which yielded an isotope infusion rate of approximately 0.12 µmole/kg·min. A prime of $NaH^{13}CO_3$ (approximately 8 mg of 80% enriched $NaH^{13}CO_3$) was given to prime the bicarbonate pool.

In each experiment the exact isotope infusion rate was calculated by first determining the dpm/mL of infusate, or the concentration of stable isotope, and then multiplying that value by the infusion rate and dividing by the weight of the dog.

Hormonal Control and Sympathetic Blockade

Somatostatin was infused through the catheter in the vena cava at the rate of 0.5 µg/kg·min to block the release of insulin and glucagon. Insulin and glucagon were replaced by intraportal

infusions at the rates of 0.2 mU/kg·min and 0.9 ng/kg·min, respectively in the control dogs. In the septic dogs, the same rate of insulin infusion was used, but glucagon was infused at 1.8ng/kg·min, because when an infusion rate of 0.9 ng/kg·min was used severe hypoglycemia developed. Combined α and β adrenergic blockade was induced by injecting propranolol (2 $mg/kg^{-1}$) and phentolamine (1.5 $mg/kg^{-1}$) simultaneously through the venous line. Forty percent of the dose was given at the start of period 2, and the remainder was given in three equal injections over the next 2h. These dosages are several fold in excess of what has been reported to be adequate in man to produce blockage (31). Our previous investigation in dogs (32) substantiated, to the extent possible, that complete adrenergic blockade was produced with this regime.

Sampling

Arterial blood samples were drawn before the start of the isotope infusion to determine baseline levels of enrichment, and throughout subsequent periods as described above. Expired air was collected through a three-way Rudolph valve attached to a cuffed endotracheal tube that was inserted through the tracheostomy on the day of the experiment. Lidocaine hydrochloride, topical anesthetic was smeared on the cuff so that when it was inflated a vagal reflex was not elicited. The expired air was collected in Douglas bags or 5 liter anesthesia bags throughout the infusion to enable the determination of $O_2$ consumption ($VO_2$), $CO_2$ production ($VCO_2$), $^{14}CO_2$ specific activity and $^{13}CO_2$ enrichment. Arterial blood pressure was determined by means of a Statham pressure transducer attached to the arterial catheter, and expressed as mean arterial pressure.

Analysis of Samples

Blood samples were kept on ice until centrifuged at 4°C to separate the plasma. A duplicate determination of glucose concentration was made on all plasma samples with a glucose autoanalyzer (Beckman, Inc., Fullerton, CA). Plasma to be used for the determination of glucagon concentration was transferred immediately to prechilled tubes containing aprotinin and Na EDTA. Insulin and glucagon concentrations were analyzed by radioimmunoassay (33, 34).

Glucose Specific Activity

Plasma glucose specific activity (SA) was determined by first precipitating the plasma proteins with $Ba(OH_2)$ and $ZnSO_4$ and then passing the resultant supernatant through anion (Dowex AG1-X8) and cation (Dowex AG 50W-X8) exchange columns. The

glucose concentration of the eluate was measured in duplicate with the glucose autoanalyzer. The remainder of the eluate was pipetted into vials, in duplicate, dissolved in 1.0 mL of water and counted in 10 mL of Hydrofluor (National Diagnostics, Parsippany, NJ) using a liquid scintillation counter (Searle Mark III).

Palmitate Enrichment

Plasma (0.5 to 1.0 mL was processed for quantitative gas chromatography (GC) as described by McDonald-Gibson and Young (35). The enrichment of the palmitic acid methylester was determined on a gas-chromatograph mass-spectrometer (Hewlett Packard 5985B). GC separation was performed on a 3" x 2 mm glass column packed with 5% DEGS on 100-200 mesh supelcoport, with a column temperature of 190°C, and the helium carrier gas flow rate was 30 mL/min. Electron impact ionization was used and ions at m/e 270.2 and 272.2 were selectively monitored in order to determine isotopic enrichment (36).

Expired Air

The expired breath for $VO_2$ and $VCO_2$ determinations were collected over 5 minute intervals. The total volume expired (STDP) was determined on a volumeter (W. E. Collins, Braintree, MA), and the concentrations of $O_2$, $N_2$ and $CO_2$ were determined on a Perkin-Elmer 1100 Medical Gas Analyzer. The method described by O'Keefe, et al (36) was used to determine the $^{14}CO_2$-SA of expired air. This involved the trapping of a known amount of $CO_2$ (1 mmole) in a hydroxide of Hyamine solution to which phenolphthalein was added to indicate when the solution was saturated with $CO_2$. Fresh solution was made each day and checked to titration with 0.15 N HCl. In addition, a calibrated gas mixture of $^{14}CO_2$ (Matheson), was used to ensure the accuracy of the method. For the determination of $^{13}CO_2$ enrichment, approximately 4 liters of expired air was first bubbled through 15 mL 0.1 NaOH for 2 min (using a vacuum pump) to trap the $CO_2$. The trapped gas was liberated in an evacuated Rittenberg tube by the addition of concentrated (85%) phosphoric acid and the atom percent excess (APE) as compared to a tank standard was then determined on a dual-inlet, dual-collector isotope ratio mass spectrometer (Nuclide model 3-6-RMS) (30).

Calculations

Plasma kinetics of all substrates were calculated according to the general principle of the Steel equation (37). Because good plateaus in plasma and expired $CO_2$ enrichment were achieved

over the last 30 min of each period, data were calculated by using equations that relied on the existence of an isotopic steady state. This approach avoided the problem in data interpretation that would have arisen owing to differences in volume of distribution after sepsis.

Rate of Appearance

In the isotopic steady state, defined as the situation in which specific activity or enrichment is not changing with time, the rate of appearance (Ra) of the substrate is:

$$(1)\quad Ra\ (\mu mole/kg \cdot min) = \frac{F}{SA \text{ or } IE}$$

where F is isotope infusion rate and SA is specific activity and IE is isotopic enrichment (for stable isotopes). When stable isotopes were used, a correction was made for the isotope infusion rate (which contributes as much as 2% to the endogenous turnover). In this case:

$$(2)\quad Ra\ (\mu mole/kg \cdot min) = \left(\frac{IE_i}{IE_p} - 1\right) \times F$$

where $^{IE}i$ and $^{IE}p$ are the isotopic enrichment of the infusate and the substrate in plasma, respectively.

At a steady state:

$$(3)\quad \text{Uptake} = Ra$$

Glucose Oxidation

$$(4)\quad \text{Glucose oxidation} = \frac{SA^{14}CO_2 \times VCO_2}{SA\ Glucose \times k}$$

where $SA^{14}CO_2$ and SA glucose are specific activity of expired $CO^2$ and plasma glucose respectively, and k is the bicarbonate recovery factor. Previous experiments with $NaH^{14}CO_2$ infusions indicate k = 1 in our dog model.

$$(5)\quad \%\ VCO_2 \text{ derived from glucose} = \frac{SA^{14}CO_2 \times 6}{SA\ Glucose}$$

(6) % Uptake oxidized of glucose
= $\%VCO_2$ from glucose/glucose uptake.

Palmitate Oxidation

(7) % of $VCO_2$ from one carbon of palmitate = $\frac{IE_{CO_2}}{IE_p \times 2}$

(8) Plasma palmitate oxidation (μmole/kg·min) = % $VCO_2$ from 1 C of palmitate x $VCO_2$

(9) % of $VCO_2$ coming from palmitate = $\frac{\text{Palmitate oxidation} \times 16\ CO_2/\text{palmitate}}{VCO_2} \times 100$

Statistical Analysis

The values in period 1, 2, and 3 were compared in each group and the values for each period in control animals were compared to the corresponding values for the same period in septic animals. Statistical comparisons between periods in the same group were made using the analysis of variance and Newman-Keuls post-hoc test. The paired Student's t-test was used for comparison of control versus septic responses in the same period.

## B. EFFECT OF SEPSIS ON VERY LOW DENSITY LIPOPROTEIN KINETICS: RESPONSES IN BASAL STATE AND DURING GLUCOSE INFUSION

### METHODS

Animals

All studies were performed in conscious unrestrained dogs with arterial, venous and portal catheters chronically in place, and with tracheostomies, which were prepared as described previously (27). The dogs were allowed 7-10 days to recover from their surgical procedures, during which time they were fed regular dog chow and canned dog food. After their recovery, studies were performed once with the dogs in the control state and once after induction of sepsis. All studies were performed in accordance with the guidelines for the use and care of laboratory animals as promulgated by the American Physiological Society and National Institutes of Health, and approved by the Animal Use Committee of the Shriners Burn Institute and of the U. S. Army.

Assessment of body temperature, heart rate, cardiac output and general appearance indicated that the dogs were in good health at the time of the control study. After the control study,

they were fed and were given antibiotics prophylactically (500 mg ampicillin, intravenously). They were allowed to rest for 2-3 days before sepsis was induced. Sepsis was induced by the infusion of live E. coli, as described above.

Experimental Design

A total of 22 studies were performed in 11 dogs. All dogs had been fed in the morning on the day before the study, so all had fasted for 24 h at the time of the study. In all studies, the experiments were conducted in three periods. Tracer doses of $^{3}H$, $^{14}C$-VLDL, and 1-$^{13}C$-palmitate bound to albumin were infused continuously throughout all three periods (see below). Periods 1 and 2 were carried out in the same manner in all studies.

In period 1, which lasted for 2 h, only isotopes were infused. Over the first 90 min, breath samples were collected over 5-min intervals to determine oxygen consumption ($VO_2$) and carbon dioxide production ($VCO_2$). Breath was collected through a three-way valve attached to a cuffed endotracheal tube inserted through the tracheostomy on the day of the study. Local anesthetic (Lidocaine) was smeared on the tube, so the animal was not disturbed at all by the collection of breath. Four blood samples were collected at 10-min intervals between 90 and 120 min to determine basal plasma kinetics, and samples of expired breath were collected at the same time to determine $^{13}CO_2$ and $^{14}CO_2$ enrichment. After the 120-min sample had been collected, and without interruption of the isotope infusion, somatostatin was infused into the peripheral circulation to inhibit the secretion of insulin and glucagon, and then the hormones were replaced by intraportal infusion (period 2). The infusion rates were designed to maintain normal hormone concentrations and thus plasma kinetics (see Infusions, below).

Period 2 lasted for 60 min. Blood samples were taken at 45, 52 and 60 min, and expired air was collected over this period also. At the end of period 2, one of two protocols was followed for period 3, which lasted for 2 h.

In one protocol used during period 3 (5 dogs), a constant glucose infusion was given at a rate of 7 mg/kg·min, while insulin and glucagon levels were maintained at the same levels as in period 2. Samples were taken at 30, 60, 90, 105 and 120 min in period 3. Breath samples were collected for $^{13}CO_2$ and $^{14}CO_2$ determinations at the same time as the blood samples. $VO_2$ and $VCO_2$ were determined between the times that blood samples were taken.

At the start of period 3 in the second protocol (6 dogs), the rate of infusion of insulin was increased to four times that used during period 2. The blood glucose concentration was tested every 10 min., and enough glucose was infused to maintain the same concentration as in period 2. The average rate of infusion needed to accomplish this was approximately 4 mg/kg·min. Samples were collected throughout period 3 as in the other protocol.

One protocol, therefore, tested the effect of hyperglycemia in the absence of an insulin response, and the other protocol tested the response to hyperinsulinemia in the absence of hyperglycemia. Each dog was tested by the same protocol before and after sepsis.

Infusions

The doubly-labeled VLDL was synthesized in vivo because none is available commercially to use as a tracer: 1-$^{14}$C-palmitate and 2-$^{3}$H-glycerol were infused into donor dogs and the labeled VLDL they produced was isolated. Most of the label is incorporated into the VLDL-TG by this procedure, although it is possible that up to 10% was actually incorporated into phospholipids. We have assumed this fraction to not affect our conclusions regarding VLDL kinetics.

Albumin (Fraction V, Arnel Products Co.) was labeled with 1 mCi of 1-$^{14}$C-palmitate (New England Nuclear, Boston, MA) before infusion and 2-$^{3}$H-glycerol (1.5 mCi) was then added. The mixture was infused (over a period of 5 min), through a catheter in the portal vein, into dogs anesthetized with sodium pentobarbital. Twenty-five minutes later, blood was collected through two arterial catheters and transferred to heparinized tubes, which were placed on ice until the plasma was separated out in a refrigerated centrifuge (Sorrel RC-5). The plasma samples were pooled before labeled VLDL was isolated.

To isolate the VLDL, 25 mL of the dog plasma was pipetted into Beckman No. 326823 polyallomer ultracentrifuge tubes. By careful application with a syringe, 6 mL of 0.15 N NaCl solution (d = 1.005) was layered on top of the plasma. The tubes were balanced and placed in a Beckman 50.2 Ti rotor and spun in a Beckman L2-65 ultracentrifuge at 34,000 rpm for 20 h at 15°C. After ultracentrifugation, the top layer containing the VLDL was separated. The isolated VLDL was pooled and concentrated. An aliquot of the VLDL-TG was taken for a subsequent test to verify the purity, from which the lipid was extracted with chloroform-methanol (2:1,V/V). (See below).

The concentrated VLDL solution for infusion was dissolved in approximately 2 mL of a 25% albumin solution and then was added to 12 mL of a 5% albumin solution, containing approximately 2 μmole/mL of 1-$^{13}$C-palmitic acid. The palmitic acid (Merck Isotopes, Montreal, Canada) was bound to the albumin by a process that involved making a potassium salt of the palmitate, as described previously (30). The bicarbonate pool was primed with 5.1 μmole/kg of $NaH^{13}CO_3$ and 7 x $10^5$ dpm/kg of $NaH^{14}CO_3$. The isotope mixture was infused into the dogs at 0.764 mL /min by using a Harvard syringe pump, which resulted in isotope infusion rates as follows: $^3$H-VLDL, 1.2 x $10^4$ dpm/kg·min; $^{14}$C-VLDL, 1.5 x $10^4$ dpm/kg·min and 1-$^{13}$C-palmitate, 0.10 μmole/kg·min.

The principal reason to be concerned about the purity of the labeled VLDL infusion was to be sure that no $^{14}$C-palmitate was infused that was not in the VLDL fraction. This was ensured by placing an aliquot of lipid extracted from the infusate on a Sephadex LH-20 column with chloroform and methanol (2:1 v/v), and collecting the eluant in fractions. In one case, only the labeled TG was put on the column, and in the second case, 1-$^{14}$C-palmitate was also added. The TG eluted as a single peak that was distinct from the $^{14}$C-palmitate peak (Fig. 1). In addition to this test, a sample of the infusate was tested for purity by thin layer chromatography (Silica gel). A single band corresponding to the triglyceride standard and distinct from the palmitate standard, was observed. When the remainder of the plate was scrapped and counted, minimal counts were detected.

## Hormonal Control

Somatostatin was infused through the catheter in the vena cava at the rate of 0.5 μg/kg·min to block the release of insulin and glucagon. Insulin was replaced in the control dogs by intraportal infusion at the rate of 0.2 mU/kg·min and glucagon was replaced at 0.9 ng/kg·min. In the dogs with sepsis, the same rate of infusion was used for insulin, but glucagon was infused at 1.8 ng/kg·min, because at 0.9 ng/kg·min, severe hypoglycemia developed. The insulin infusion was increased to 0.8 mU/kg·min in period 3 of the protocol to test the effect of hyperinsulinemia.

## Sample Analysis

Blood samples were kept on ice until they were centrifuged, at 4°C, to separate the plasma. Determinations of plasma glucose were made in duplicate by using a glucose autoanalyzer (Beckman, Inc., Fullerton, CA). The plasma to be used for the determination of glucagon concentration was transferred immediately to prechilled tubes that contained aprotinin and Na EDTA. Insulin and glucagon concentrations were analyzed by radioimmunoassay.

Palmitate was extracted from plasma and isolated by thin layer chromatography (TLC) and the enrichment of the palmitic acid methyl ester was determined by gas-chromatography mass-spectrometry (GCMS), as we have described (30), with a Hewlett-Packard 5985B GCMS system. Palmitic acid and total FFA concentration were quantified on a Varian 2100 gas chromatograph and CDS 111 Data System using heptadecanoic acid as an internal standard. Specific activity was determined by scintillation counting after palmitate was isolated by TLC.

The VLDL-TG was isolated from the plasma by placing 1 mL aliquots of plasma into No. 331370 cellulose nitrate centrifuge tubes and topping off the plasma with 10 mL of a .15 N NaCl solution (d=1.005). The samples were spun in a Beckman L2-65B ultracentrifuge at 29,500 rpm (109,000 g) for 20 h at 15°C with a Beckman SW41 rotor. After ultracentrifugation, the top layer containing the VLDL-TG was carefully removed with a roller aspirating pump. The triglyceride concentration in the solution containing the VLDL was measured by using a kit (Harleco Tri-ES Kit), and a measured volume of the solution was counted, with Hydrofluor (National Diagnostic, Parsippany, NJ) as scintillant. A Searle Analytic Beta Counter was used with the dual isotope program.

The expired gas for $VO_2$ and $VCO_2$ determinations was collected over 5-min intervals. The total volume expired (STPD) was determined on a volumeter (W.E. Collins, Braintree, MA), and the concentrations of $O_2$, $N_2$ and $CO_2$ were determined on a Perkin-Elmer 1100 Medical Gas Analyzer. The method described by O'Keefe, et al., was used to determine the $^{14}C$-SA of expired air. One mmole of $CO_2$ was trapped in a hydroxide of Hyamine solution to which phenolphthalein was added to indicate when the solution was saturated with $CO_2$. To measure the isotopic enrichment of $^{13}CO_2$, approximately 5 liters of expired air was bubbled through 0.1 N NaOH to trap the $CO_2$. The trapped gas was liberated in an evacuated Rittenberg tube by adding concentrated (85%) phosphoric acid, and the atom percent excess (APE) was then determined on an isotope-ratio mass-spectrometer (dual-inlet, dual collector) (Nuclide model 3'-60°RMS), as we described earlier (38).

## Calculations

Calculation of the rate of substrate oxidation during a constant isotope infusion requires a physiological and isotopic steady state for interpretation of the data to be reliable (39). A steady state was achieved in periods 1 and 2 of all studies, so oxidation rates were calculated in the basal state in both control and septic dogs. During period 3, however, in both

protocols, the enrichment of expirated $CO_2$ often did not reach a new equilibrium value by the end of the period. As a consequence, oxidation rates were not calculated for period 3. The enrichment of plasma VLDL and plasma FFA, on the other hand, did reach new isotopic equilibriums in period 3. In most cases, this had been achieved at the end of 60 min of period 3, but in all cases, it had by 90 min. Therefore, the plasma kinetic measurements in period 3 were based on the average enrichments among the samples obtained between 90 and 120 min of the period.

The total plasma flux of FFA was determined by means of the $^{13}C$-palmitate data. The calculation of the contribution of fatty acids released from VLDL to the total plasma FFA flux was based on the $^{14}C$-specific activity (SA) of the plasma palmitate. The VLDL kinetic data were calculated from the $^{3}H$-VLDL data. By using these approaches, we were able to calculate the extent to which the fatty acids in VLDL were directly taken up into tissues and oxidized to $CO_2$ (as opposed to entering the plasma pool first). This information was important to help clarify the physiological significance (in terms of energy metabolism) of regional changes in lipoprotein lipase activity in different physiological states.

The equation to calculate the plasma flux of FFA and the rate of FFA oxidation from the 1-$^{13}C$-palmitate date has been described and discussed in detail previously (30).

The following calculations were used to calculate VLDL kinetics and oxidation:

(1) Rate of appearance of VLDL (RaVLDL) into plasma (μmole/kg·min

$$= \frac{^{3}H \text{ infusion rate (dpm/kg·min)}}{\text{VLDL SA (dpm/μmole TG)}}$$

where SA is specific activity.

(2) Ra of $^{14}C$-FFA in plasma (dpm/kg·min

= Plasma FFA-SA (dpm/μmole) x total RaFFA (from $^{13}C$-palmitate data)

(3) Ra of FFA derived from VLDL (μmole/kg·min)

$$= \frac{\text{Ra of } ^{14}C\text{-FFA (dpm/kg·min)}}{\text{SA of FFA in infusate (dpm/μmole fatty acid)}}$$

(4) Fraction of plasma FFA derived from VLDL

$$= \frac{\text{Ra of FFA from VLDL (μmole/kg·min)}}{\text{Total RaFFA from } ^{13}\text{C-data}}$$

(5) Total rate of oxidation of fatty acids in VLDL (μmole/kg·min)

$$= \frac{^{14}CO_2 \text{expired (dpm/kg·min)}}{^{14}\text{C infusion rate} \times 0.95 \text{ (dpm/kg·min)}} \times \text{RaVLDL-TG} \times 3 \text{ (μmole FA/μmole VLDL-TG)}$$

The 0.95 is to correct for bicarbonate retention, determine from $NaH^{14}CO_3$ infusions.

(6) Oxidation of plasma FFA derived from VLDL

$$= \frac{\text{RaFFA from VLDL (μmole/kg·min)}}{\text{Total RaFFA (from } ^{13}\text{C-data)}} \times \text{total plasma FFA oxidation rate (μmole/kg·min)}$$

Since the total rate of oxidation of the fatty acids in VLDL (5) is equal to the sum of the oxidation of the plasma FFA that would have been derived from the VLDL and the fatty acids from VLDL that have been directly oxidized without entering the plasma pool, then:

(7) Direct oxidation of VLDL fatty acids (μmole/kg·min)

= (6) - (5).

## Results

### A. EVALUATION OF THE ROLE OF GLUCAGON AND INSULIN ON GLUCOSE AND FFA KINETICS IN SEPSIS

The basal concentrations of insulin and glucagon were significantly higher ($P<0.01$) in septic animals than in controls (Table 1). When somatostatin, insulin and glucagon were infused at constant rates, there was no significant change from period 1 to period 2 in the plasma insulin concentration in either the control or septic animals. The plasma glucagon concentration also did not change significantly in the control group. In contrast, the glucagon level decreased significantly during the infusion in the septic dogs (Table 1). Thus, hormonal control selectively reduced the plasma glucagon level in septic

animals, but had no effect on the insulin or glucagon levels in non-septic animals. Adrenergic blockade during hormonal control did not affect the concentration of insulin or glucagon, (Table 1).

The behavior of the control dogs was not changed by either the procedure for controlling the hormones or the initiation of adrenergic blockade. In contrast, both procedures were stressful to the septic animals. The septic animals became lethargic after the hormones were controlled, and their lethargy increased when sympathetic activity was blocked. These effects were probably the result of the progressive decrease in the plasma glucose level that followed these perturbations (see below) as blood pressure did not change significantly (100±5 mm Hg before hormonal control plus sympathetic blockade and 90±10 mm Hg after).

When the levels of insulin and glucagon were controlled at the basal level in the non-septic animals there was no significant change in plasma levels of glucose or palmitate. The kinetics and oxidation of these substrates also remained constant. In contrast, the control of insulin and glucagon levels in the septic animals produced pronounced metabolic changes.

The basal rate of glucose production was similar in the septic animals and in the controls. However, whereas institution of hormonal control had no significant effect on glucose kinetics in the control animals, it caused a significant reduction in the rate of glucose production in the septic dogs, so that the plasma glucose concentration fell to 2.7 ± 0.3 μmole/mL which was significantly lower than the corresponding control value of 6.0 ± 0.5 μmole/mL (Fig. 2). When sympathetic blockade was added to hormonal control, the rate of glucose production and plasma glucose concentration rose significantly in the controls ($P<0.01$), whereas in septic animals glucose production was decreased further ($P<0.05$), as was the plasma glucose concentration (Fig. 2). Thus, although the basal rate of glucose production in septic animals was not significantly different than in controls, during adrenergic blockade and normalization of insulin and glucagon levels, the rate of glucose production in septic animals was reduced to 7.0 ± 1.5 μmole/kg·min, as compared to the corresponding value in control dogs of 19.5 ± 1.5 μmole/kg·min.

Changes in glucose oxidation generally paralleled the changes in glucose production and uptake. Since the rates of both $VCO_2$, as well as glucose uptake varied among the different groups of dogs and protocols, we have expressed the glucose oxidation data as the % $VCO_2$ from glucose per unit of glucose uptake. Differences in $VCO_2$ and in glucose uptake are factored out by this approach so that a figure is derived that reflects the ability of the body to oxidize a given amount of glucose in relation to the level of energy expenditure. In the control

animals, no change in this figure was observed in any protocol. In the septic animals, the value was not significantly different from the control value either in the basal state or when insulin and glucagon were controlled. However, the addition of adrenergic blockade to hormonal control in septic animals caused a significant increase in the % $VCO_2$ from glucose expressed as a function of glucose uptake, thereby raising the value above the corresponding control value (Fig. 2).

The plasma palmitate concentration, the rate of appearance of palmitate, the contribution of palmitate to the $VCO_2$, and the rate of palmitate oxidation were all significantly higher ($P<0.01$) in the septic animals than in the controls. When the hormones were controlled, there was no significant change in either the rate of turnover of oxidation of palmitate in any of the dogs. The addition of adrenergic blockade to hormonal control, however, produced a significant decrease in the rate of appearance of palmitate in both groups. The plasma palmitate concentration decreased significantly in the control animals, but the decrease in the septic group did not reach statistical significance. The % of $VCO_2$ derived from palmitate did not change significantly following either hormonal control or sympathetic blockade in either the septic or non-septic groups, but the absolute rate of palmitate oxidation fell during adrenergic blockade in the septic animals in conjunction with a fall in overall metabolic rate, and thus, total $VCO_2$ (Fig. 3). When the % of $VCO_2$ was expressed as a fraction of the rate of palmitate uptake, there were no significant differences between septic and control animals.

## B. EFFECT OF SEPSIS ON VERY LOW DENSITY LIPOPROTEIN KINETICS: RESPONSES IN BASAL STATE AND DURING GLUCOSE INFUSION

### RESULTS

#### Effect of Sepsis on Basal Kinetics

The data from period 1 of both protocols were pooled to determine the effect of sepsis on basal kinetics. Sepsis was found to cause a significant increase in the concentration of plasma TG and RaVLDL, but the magnitude of the increase in RaVLDL was much greater than the magnitude of that of TG concentration (Table 2). Similarly, the concentration and the appearance of FFA in plasma changed significantly in dogs with sepsis, but the magnitude of these increases were more in proportion with one another than were the increases in TG concentration and flux (Table 2).

The VLDL oxidation was affected by sepsis even more dramatically than was VLDL flux (assessed with $^{3}H$) (Table 3). In the controls, the oxidation of fatty acids in VLDL accounted for a minimal percentage of the total $VCO_2$ (1.6 ± .72%). In sepsis, however, it increased to 17.2 ± 5.02%, which represented a 12-fold increase in the rate of VLDL oxidation (Table 3). The $^{14}C$-palmitate specific activity in plasma was at such a low level that greater than 95% of the VLDL-fatty acids must have been taken up directly into cells, rather than entering the plasma FFA pool, before oxidation. The rate of plasma FFA oxidation also increased in sepsis (Table 3), with the result that the total percent $VCO_2$ attributable to the oxidation of plasma fatty acids (VLDL and FFA) increased from 18% in the control dogs to 45.6% in the dogs with sepsis.

Effect of Hormonal Clamp

In the control dogs, the insulin concentration was 13.1 ± 2.1 μU/mL in period 1, and 12.6 ± 1.8 μU/mL in period 2, during hormonal control. The basal glucagon was also matched well between period 1 and period 2 during hormonal control in the control dogs (148.5 ± 45.0 versus 139.1 ± 30.6 pg/mL).

The insulin levels in dogs with sepsis were comparable to the control values in periods 1 and 2 (15.6 ± 3.2 μU/mL in period 1 and 16.5 ± 2.1 μU/mL in period 2). The basal glucagon concentration, on the other hand, was increased in dogs with sepsis, to an average value of 1002.5 ± 142 pg/mL. It decreased to 255.5 ± 41 pg/mL during hormonal control (period 2). This was significantly higher ($P<.05$) than the corresponding control value.

The hormonal control produced no metabolic effects in the control dogs. In the dogs with sepsis, lowering the glucagon concentration decreased the plasma glucose concentration significantly. The rate of glucagon infusion had to be twice that used in the controls in order to avoid hypoglycemia. Despite the effect of lowering the glucagon level on glucose concentration in dogs with sepsis, none of the factors related to VLDL or FFA kinetics or oxidation were affected significantly.

Insulin and/or Glucose Infusion (Period 3)

In both groups, the goal of the experimental design was met. Increasing the rate of infusion of insulin in period 3 caused an increase in the plasma concentration to approximately 40 μU/mL in both the control dogs and those with sepsis (Fig. 4). In both cases, a normal glucose

concentration was maintained by infusing glucose at a rate that varied as required (Fig. 4). Conversely, when the glucose was infused with the insulin maintained at a constant infusion rate, the blood level of glucose increased significantly, and the plasma insulin level did not change significantly (Fig. 4). Control of hormone levels in the dogs with sepsis was not always complete; in a few dogs, the insulin level rose in period 3 despite the relatively high rate of infusion of somatostatin. Even in those dogs, however, the insulin levels were well below what was reached during insulin infusion at .8 mU/kg·min.

Hyperglycemia caused an increase in RaVLDL in control dogs, from 0.31 ± 0.08 μmole/kg·min to 0.65 ± 0.12 μmole kg·min (statistically significant, $P<.05$), but hyperinsulinemia did not change it significantly (Fig. 5). The plasma triglyceride concentration fell slightly (0.63 ± 0.11 to 0.57 ± 0.09 μmole/mL) with the hyperinsulinemia, and increased slightly (0.48 ± 0.07 to 0.54 ± 0.07 μmole/mL) with hyperglycemia, but neither change was statistically significant. In the dogs with sepsis, neither change was statistically significant. In the dogs with sepsis, neither hyperglycemia nor hyperinsulinemia stimulated RaVLDL significantly (Fig. 5).

Both hyperglycemia and hyperinsulinemia caused a reduction of more than 50% in plasma FFA concentration and RaFFA, in control dogs and dogs with sepsis alike (Fig. 5). RaFFA in the dogs with sepsis during period 3 (both protocols) remained significantly ($P<.01$) elevated above the corresponding control value.

The importance of RaFFA as a determinant of RaVLDL is well recognized (25). Since sepsis caused a pronounced increase in RaFFA and since both hyperglycemia and hyperinsulinemia caused significant reductions in RaFFA, it is useful for the purposes of this experiment to express RaVLDL in relation to RaFFA (Table 4). The ratio of RaVLDL/RaFFA was significantly higher in the dogs with sepsis than the control dogs in the basal state. During hyperglycemia in control dogs, the ratio rose up to a value that corresponded with those in the dogs with sepsis. The increase in the ratio during hyperinsulinemia in the control dogs was also significant, but it increased less than during the hyperglycemia. The increases in period 3 in the dogs with sepsis were not statistically significant.

## DISCUSSION

### A. ROLE OF INSULIN AND GLUCAGON IN SUBSTRATE KINETICS IN SEPSIS

In this septic dog model, the animals are generally in a hyperdynamic cardiovascular state in which they undergo changes in many hormonal and metabolic factors comparable to those seen in humans in sepsis (27). The rates of glucose production and the appearance of fat are consistent with reports from studies in patients (5, 17). Similarly, the substantial increase in plasma glucagon concentration we observed has been reported to occur in humans (40). Since the basal insulin level was not increased as much as the glucagon level in sepsis, we were able to selectively decrease the concentration of glucagon in the septic dogs toward the control levels.

The fall in glucose production that occurred in septic animals when glucagon was decreased toward the normal level indicates the importance of chronic hyperglucagonemia in maintaining glucose production in sepsis. These results may seem somewhat unexpected because of findings which indicate that the effect of glucagon infusion on glucose production is transient in normal dogs, although the effect can be prolonged if the infusion is given with epinephrine (41); and that a resistance to the stimulation of hepatic c-AMP content by glucagon occurs during sepsis (42). Nevertheless, the role of hyperglucagonemia in maintaining glucose homeostatis in this septic model is undeniable. It is likely that the hyperglucagonemia in the septic dogs resulted from a generalized stress response rather than as a counterregulatory response to early hypoglycemia, since in fact the early response to gram-negative bacterial infusion is hyperglycemia (1). We could not evaluate the effects of lowering the glucagon levels completely to the control level in the septic dogs because, in a pilot study, with two septic animals, when the glucagon concentration was decreased to below 200 pg/mL the animals became profoundly hypoglycemic, and were quickly revived by glucose infusion. In this experiment, therefore, we infused glucagon at a rate sufficient to maintain glucagon levels close to 300 pg/mL yet the dramatic effect on glucose production was still observed. Because during normal control the glucagon level was still higher in the septic dogs than in the nonseptic dogs, it was not possible to estimate the fraction of basal glucose production for which glucagon was responsible. That fraction, however, is likely to be at least as high as one-third, the value reported for normal dogs (43).

In addition to lowering the insulin and glucagon level, somatostatin inhibits growth hormone secretion. Throughout all period involving somatostatin infusion, therefore, there existed a growth hormone deficiency. We have performed our previous studies in this model replacing growth hormone in some cases (32,44), and not replacing it in others (28). The nature of acute responses of glucose and palmitate metabolism to perturbations is not normally affected by the replacement of growth hormone. In a pilot study for this experiment, inclusion of growth hormone in the infusion made no difference in the observed responses.

Our results also indicate that the role of the SNS in maintaining glucose homeostastis may be altered in septic dogs. Normally, the basal sympathetic activity has little direct effect (32). We have shown in earlier studies that the increase in glucose production after adrenergic blockade during hormonal control occurs because of concommitant changes in palmitate concentration; thus, if changes in palmitate concentrations are prevented, glucose production is not affected by adrenergic blockade (28). In contrast, in this study, adrenergic blockade caused a significant decrease in glucose production when given to septic dogs during control of hormones, which suggests the existence of a pre-existing stimulatory role for the sympathetic nervous system. Extrapolation of these data to the effects of the basal sympathetic activity on glucose production in sepsis is not possible, however, because the hormonal control had already caused a pronounced hypoglycemia before the blockade was induced and that hypoglycemia itself might have elicited a sympathetic response that was greater than basal. Despite the interpretative limitations imposed by the circumstances in which the role of sympathetic activity was assessed, we elected to use this approach because of the changes in glucagon and insulin that would have occurred after blockade without hormonal control (32), which would have complicated the interpretation of the data even more. In any case, we achieved clear evidence that when glucagon levels were normalized in sepsis, sympathetic activity played a role in stimulating glucose production. However, in this setting the sympathetic effect was not sufficient to enable the septic dogs to maintain a basal rate of glucose production and plasma glucose concentration in the absence of the stimulus of the pre-existing high glucagon level.

Since the combination of hormonal control and blockade decreased glucose production in septic dogs but increased glucose production in control dogs, we can possible reconcile some apparently contradictory findings from <u>in vivo</u> and <u>in vitro</u> experiments on the effects of sepsis on glucose production. We

propose that hormonal control with SNS blockade enabled us to detect in vivo the underlying inhibitory effect of sepsis on glucose production that has been reported in vitro studies (1, 3), and that in vivo counterregulatory responses in septic animals can overcome the hepatic defect in glucose production. Thus, the rate of glucose production in sepsis depends upon the balance between the direct inhibitory effect of sepsis on gluconeogenic enzymes (4) and the stimulatory effects of hyperglucaconemia and perhaps increased sympathetic tone. An alternative interpretation of the data is that although the ability of the chronic hyperglucagonemia to stimulate glucose production above the normal rate waned, the basal rate of glucose production became dependent on the high level of glucagon. This possibility is supported by studies in which glucagon was infused and even though glucose production returned to normal during the glucagon infusion, when the glucagon infusion was stopped, glucose production fell below normal (45). The difference between the glucagon infusion experiments and this experment is that the glucagon infusion caused hyperglycemia, which would have served to inhibit glucose production as soon as the glucagon infusion was stopped, whereas in this study, there was no hyperglycemia in the dogs with sepsis.

Unlike the analysis of the rate of glucose production, the interpretation of the glucose oxidation data may differ according to the means by which the data are expressed. The rate of glucose oxidation can vary according to the rate of appearance and uptake of glucose, as well as the overall metabolic rate. In groups in which either or both of these factors differ, the absolute rate of glucose oxidation probably will not provide an accurate reflection of the ability of each group to oxidize a given amount of glucose. Thus, in order to assess the effect of sepsis, as well as hormonal control, on the oxidation of glucose by the whole body, we chose to factor out differences in both $VCO_2$ and glucose uptake by expressing the data as the % $VCO_2$ from glucose/glucose uptake. When expressed in this manner, our results indicated that the septic animals were able to oxidize about the same amount of glucose as the controls. Furthermore, the data from both control and septic dogs indicated that glucagon played no direct role in controlling the rate of glucose oxidation, apart from affecting production and thus uptake. Similarly, sympathetic activity seemed to have no direct role in glucose oxidation in normal animals. Whereas the data presented in Fig. 1 suggests that sympathetic activity had a depressive effect on glucose oxidation in septic animals, it should be kept in mind that when the septic dogs received hormonal control plus blockade, their rates of glucose production and uptake were extremely low. It is likely that at very low rates

of glucose uptake any linearity in the relationship between glucose oxidation and uptake would be disrupted because a large portion of available glucose would be taken up by the brain, which oxidizes a large portion of its glucose uptake (46). At rates of glucose uptake greater than basal, studies involving the quantitation of glucose oxidation during glucose infusion indicate that oxidation rises roughly in proportion to the rate of uptake until the glucose infusion approaches total caloric requirement (47).

In septic dogs, both the basal rates of appearance of palmitate and the oxidation of plasma palmitate were high. The increased rate of appearance of plasma palmitate in sepsis was attributable, at least in part, to increased sympathetic activity; the hyperglucagonemia of sepsis did not appear to be responsible for this occurrence. These findings are not surprising because the SNS is known to be a potent stimulant of lipolysis (16) and increased catecholamine activity has been documented extensively in the stress state (48). This response of palmitate kinetics is similar to that produced in burn injury. When adrenergic blockade was administered to burned animals with insulin and glucagon controlled as in this experiment, the elevated rate of appearance of palmitate also decreased significantly (44). Since the ability of the septic animals to oxidize the palmitate was not impaired, the increased availability of the palmitate produced an increase in the importance of plasma palmitate (and presumably other FFA) as an energy substrate in sepsis.

### B. EFFECT OF SEPSIS ON VERY LOW DENSITY LIPOPROTEIN KINETICS: RESPONSES IN BASAL STATE AND DURING GLUCOSE INFUSION

In this experiment, intravenous infusion of live *E. coli* caused a significant increase in plasma TG concentration. This response has been observed in many previous studies, in dogs and in other animal models, as well as in human patients (e.g., 49, 50, 51). In fact, hypertriglyceridemia could be considered to be a hallmark of sepsis (52). VLDL kinetics, however, have not been investigated previously in either a model of sepsis or in human patients. In this model of *E. coli*-sepsis in dogs, we have shown that the increase in TG concentration is due to an increased rate of production of VLDL rather than a decreased rate of TG clearance.

The increase in plasma TG concentration in gram-negative sepsis was ascribed previously by Kauffman and associated to impaired TG clearance from plasma. Their conclusion was based on the observation that in monkeys injected with either live bacteria or endotoxin, the TG clearance rate was reduced after

injection of a lipid emulsion and also that the lipolytic activity in plasma after administration was lower than in control animals (51). The decrease in lipolytic activity ordinarily caused by administration of heparin was assumed to be indicative of a decrease in the activity of lipoprotein lipase (LPL), the enzyme responsible for the hydrolysis of plasma triglycerides. Extrapolation from the results of these studies, however, is limited because the mechanisms involved in clearing the TG from plasma when they are present in the extremely high concentrations that resulted from the injection of intralipid may be different from the clearance of endogenously produced TG. Also, a decrease in the lipolytic activity caused by heparin does not necessarily represent a decrease in the activity of lipoprotein lipase (LPL), the enzyme responsible for the hydrolysis of plasma TG, because hepatic triacylglycerol lipase is also released into plasma by heparin (20). The apparent contradiction between our results, which show a significant increase in VLDL production in sepsis, and those of other investigators who have demonstrated regional decreases in LPL activity in sepsis or endotoxemia, probably stems from the fact that the whole-body plasma VLDL kinetics have been shown to be unrelated to the LPL activity in specific tissues (52,19). The changes in LPL activity in some tissues probably do not explain the increased level of TG in the plasma, but rather coincide with alterations in regional metabolism of TG.

The importance of VLDL-fatty acids in energy metabolism in sepsis is indicated by the dramatic increase in the percentage of $VCO_2$ that arose from their direct oxidation. The rate of VLDL oxidation, expressed in μmole fatty acid/kg·min, increased to an even greater extent in sepsis than did RaVLDL. This could be explained by a greater rate of recycling of VLDL-glycerol than VLDL-fatty acids, since the $^{14}C$-specific activity of VLDL decreased more after induction of sepsis than did the $^{3}H$-specific activity. Thus, the increase in RaVLDL in sepsis, calculated from the $^{3}H$-data, actually underestimated the extent to which new fatty acids were appearing in the plasma in VLDL and were being taken up by peripheral tissues. The increased availability of fatty acids in VLDL was responsible for a proportionate increase in their oxidation, because the dogs with sepsis suffered no impairment in ability to oxidize fatty acids (as indicated by the percent of uptake oxidized).

The importance of regional changes in LPL activity in relation to energy metabolism is indicated by the fact that few of the $^{14}C$-fatty acids in VLDL entered the plasma FFA pool before oxidation took place, but rather were taken up directly into the tissues in which the hydrolysis of the VLDL occurred. In light of the increased importance of VLDL oxidation in this

septic model, it would not be surprising if a decrease in LPL activity were found in adipose tissue, since other tissues would have a greater need for the fatty acid. In support of this speculation, Bagby and Spitzer have found that surgical trauma, thermal injury and cecal ligation all resulted in increases in LPL activity in heart and skeletal muscle (53, 54), which suggests that fatty acids are redirected to tissues whose ability to oxidize glucose may be impaired (38). In contrast to these observations, however, Bagby and Spitzer also found a decrease in heart LPL in rats injected with endotoxin (55).

Our finding that the ability to oxidize long-chain fatty acids was not impaired in dogs with sepsis is in contrast to what has been proposed previously. Based primarily on the observation of a reduction in carnitine (56), which is necessary for the transfer of fatty acyl CoA into the mitochondria, it has been proposed that a muscle-fuel deficit develops in sepsis (56). Further, it has been proposed that this energy deficit could theoretically be circumvented if energy were provided in the form of medium-chain fatty acids, whose oxidation is carnitine-independent. The provision of medium-chain fatty acids to animals with sepsis, however, has not been shown (as could be predicted on the basis of our results) to offer a nutritional benefit remarkably greater than what is derived from emulsified long-chain fatty acids (57).

The results from any experiment in which an animal model of sepsis is used cannot be extrapolated directly to humans. Nonetheless, we feel that the infusion of live bacteria into dogs, followed by fluid resuscitation, is a good model of gram-negative sepsis. Many aspects of the response are similar to what has generally been reported in patients, including increase in cardiac output, heart rate, body temperature and incidence of positive blood cultures (see Ref. 27 for detailed discussion of animal model). The plasma triglyceride concentration is elevated in this model, just as it is in patients. Because many other aspects of the metabolic response in this animal model are similar to what occurs in humans, it is not unreasonable to speculate that the same mechanisms are responsible for the increase in plasma TG in our dog model and in patients.

The results of this experiment do not provide enough information to uncover the mechanism responsible for the stimulation of VLDL production in sepsis. There are several potentially rate-limiting steps in VLDL synthesis and secretion, and they are under different controls. Nevertheless, it seems likely that the stimulation of peripheral lipolysis (with the resultant doubling of RaFFA) was a primary stimulant of VLDL production in sepsis. It has been pointed out previously that in fasting animals, the rate of VLDL production is determined

primarily by the rate of FFA flux (25). Since sympathetic activity is responsible principally for the stimulation of peripheral lipolysis in stress (58), it would follow that the sympathetic nervous system is, indirectly, causing the increased RaVLDL in sepsis. This is supported by the observation of a correlation between serum TG concentration and plasma cathecholamine concentration in canine gram-negative bacteremia (50).

The increase in RaVLDL in sepsis cannot be explained entirely by the increase in FFA flux, because the ratio RaVLDL/RaFFA was elevated significantly in dogs with sepsis. A further explanation may lie in the stimulation of phosphatidate L-α-phosphohydrolase activity by cortisol (59) which is known to be elevated in sepsis. Glucagon did not appear to play an important role in stimulating VLDL production, since the significant reduction in the glucagon level that occurred when hormonal control was undertaken (period 2) did not have a significant effect on RaVLDL. Another possibility may be that the availability of glycerol-P is limiting in the normal dogs, and the increased rate of lipolysis in sepsis provided enough additional glycerol to the liver that glycerol-P was no longer limiting.

We hoped that by testing the dynamic response of VLDL kinetics to hyperglycemia and hyperinsulinemia in both control dogs with sepsis, additional information would be obtained about the nature of the alteration in VLDL kinetics in sepsis. Indeed, a significant difference in the response to hyperglycemia was noted in the control and septic animal. The use of hormonal control enabled us to evaluate the response to hyperglycemia in the absence of an insulin response. This general approach to the separation of glucose and insulin effects in dogs was first described by Cherrington and associates (60). The use of somatostatin is helpful because it seems to have a minimum direct effect on hepatic substrate metabolism (61), yet permits the effective control of insulin and glucagon concentration. We have shown previously that physiological and metabolic steady-state conditions can be maintained over 4 h of hormonal control without other perturbation (62), so the values in period 3 in the current study could justifiably be compared with the corresponding values in period 2.

The stimulatory effect of hyperglycemia on VLDL production in vivo in the control dogs in this experiment was striking in view of the suppressive effect of the infused glucose on FFA flux, since under normal conditions, the rate of VLDL production is a linear function of the rate of delivery to FFA to the liver (25). In vitro, an increase in glucose availability causes an increase in TG synthesis (21) and further, a high carbohydrate diet will result in an increased rate of VLDL synthesis when the insulin response is blocked (63). Our study shows that in the dog the

effect of glucose infusion on RaVLDL is elicited rapidly. The ratio of RaVLDL/RaFFA reflects the increase in VLDL production that occurred relative to the supply of FFA from peripheral adipose tissue. The inhibitory effect of glucose on peripheral lipolysis, independent of insulin, has been demonstrated before (64). The ability of glucose to stimulate VLDL production, despite the drop in supply of FFA to the liver, can be attributed to the synthesis of fatty acids from the infused glucose (65).

On the basis of the results from $^{13}$C-glucose infusions in humans, we had proposed previously that a fraction of infused glucose was being incorporated into TG and oxidized as free fatty acids (66). The notion of such a pathway is consistent with a respiratory quotient (R.Q.) of 1 or greater, because the R.Q. of fat synthesis from glucose (8.7) is high enough to offset the low R.Q. of fat oxidation (0.71). Nonetheless, it is somewhat surprising to observe this response in fasted dogs infused with glucose, since our previous experimental observations were made after several days of continuous high dose glucose infusion. In the fasted state, most glucose that was not directly oxidized would be expected to be directed to glycogen rather than to fatty acid synthesis. If glucose were to be infused in the fed state, when the glycogen stores were full, the stimulatory effect of glucose infusion on RaVLDL would presumably be greater.

An alternative explanation is that the hyperglycemia did not stimulate RaVLDL through an increase in hepatic fatty acid synthesis, but rather through enhancing another rate-limiting step. Our data did not rule out this possibility, since the total fatty acids appearing in VLDL-TG were less than one-third of the RaFFA minus FFA oxidation, even though the ratio of RaVLDL/RaFFA had increased significantly. The site of a specific stimulatory action of glucose (if there is one), however, is unknown.

Whatever the mechanism by which hyperglycemia acts on RaVLDL in control dogs, it was not functioning in dogs with sepsis. Not only was RaVLDL not stimulated significantly by hyperglycemia in dogs with sepsis, but also the ratio RaVLDL/RaFFA was not significantly increased. Thus, the rate-limiting step in VLDL production in normal dogs that is stimulated by glucose is already stimulated in sepsis beyond the point at which glucose is an effective stimulus. If even a small percentage of the infused glucose that was not oxidized was converted to the fat in the liver (67), then in an absence of an increase in RaVLDL, deposition of fat in the liver would occur. This could explain the development of fatty livers in patients with sepsis, particularly during prolonged glucose infusions.

The various possibilities in the role of insulin in controlling VLDL kinetics in vivo are difficult to sort out. Even in vitro experiments have yielded contradictory results. Studies have shown that insulin increased the incorporation of labeled glucose into triglycerides and enhanced the release into medium of VLDL from liver slices (68) and from perfused rat liver (21). In a recent study, however, insulin was shown to increase TG storage and to decrease release of TG in cultured rat hepatocytes (69).

In vivo results are also difficult to interpret, because insulin usually exerts an inhibitory effect on the peripheral release of FFA, which could obscure any direct stimulatory effect on the liver. Also, since glucose infusion exerts a stimulatory effect independent of insulin, the insulin effect must be distinguished from the glucose effect. This differentiation is diffucult to make, since glucose has to be infused when the insulin concentration is elevated to prevent a change in the glucose concentration. Thus, even though euglycemia is maintained, the total amount of glucose entering the system may be increased. With these limitations in the interpretation of our data in mind, we were unable to confirm a stimulatory effect of hyperinsulinemia on RaVLDL, although hyperinsulinemia did prevent the drop in RaVLDL that would have been expected on the basis of the decrease in RaFFA. Previous studies that have led to the conclusion that insulin exerts a stimulatory effect on VLDL synthesis have either been performed in patients in whom there is a peripheral resistance to the anti-lipolytic action of insulin (24), or have used an insulin infusion rate that was much higher than the one used in our study (23). When adult onset diabetics who did not display peripheral resistance to the anti-lipolytic effect of insulin were studied, no correlation could be found between the elevated levels of insulin and the rate of VLDL synthesis (22). The difference between the results of our study and the study of Steiner, et al (23), in which VLDL production was stimulated as a consequence of a 10-fold increase in insulin concentration, could be that the maximum anti-lipolytic effect of insulin is elicited at a lower concentration than the maximum stimulatory effect on VLDL production, and we did not infuse enough insulin to achieve the maximum stimulatory effect on VLDL production, but we did inhibit lipolysis markedly. Therefore, since there was no peripheral resistance to insulin in our study, in either normal dogs or dogs with sepsis, no stimulatory effect of insulin could be detected at the dose of insulin we used.

The alterations in basal kinetics of VLDL in sepsis that we have observed are not inconsistent with commonly observed clinical responses. In particular, lipemia frequently occurs

when patients with sepsis are infused with a lipid emulsion. The kinetics of TG clearance are not linearly related to the rate of appearance at all concentrations. Rather, the removal of TG follows the kinetics of a saturable system with a Vmax of approximately 0.5 μmole/kg·min in human subjects. Our results indicate that the Vmax is higher in dogs. As long as the appearance rate of TG in plasma is below the 1/2 Vmax, one would expect modest increases in plasma concentration relative to the magnitude of increase in appearance. Once the 1/2 Vmax is exceeded, the concentration will increase at a rate disproportionately faster than that of the rate of appearance. Thus, since the basal rate of appearance of TG is much closer to the 1/2 Vmax in sepsis than it is in the normal state, an infusion of a TG-emulsion would be expected to exceed the 1/2 Vmax at a lower rate of infusion in dogs with sepsis. Thereafter further increases in the rate of infusion would cause the TG concentration to increase disproportionately faster than the increase in the rate of appearance. As a consequence, even though the basal rate of production and clearance of VLDL are enhanced in dogs with sepsis, this is not inconsistent with the development of lipemia during a lipid infusion at a rate at which lipemia would not be encountered in the control state. Thus, even though in the post-absorptive state a greater percent of $VCO_2$ originates from the oxidation of TG in sepsis than it does normally, this is not necessarily an indication that infused triglyceride is an optimal form of nutritional support.

Thus, the chief role of insulin, glucagon and the SNS in the control of glucose and palmitate oxidation involves their effect on the appearance of the substrates in plasma. The appearance of glucose in the plasma in sepsis depends upon an increase in the activity of glucagon, and probably, on sympathetic stimulation. The increase in the rate of appearance of palmitate in plasma results, at least in part, from increased activity of the sympathetic nervous system. VLDL appearance is also increased in sepsis, probably due, at least in part, to increased FFA flux. Consequently, these studies indicate that control focus should be placed on the control of energy substrate kinetics in sepsis, and that changes in substrate oxidation will occur secondarily.

## REFERENCES


1. Wolfe, R.R. and J.F. Burke. Glucose and lactate metabolism in experimental septic shock. Am. J. Physiol. 235:R219-R227, 1978.

2. Hinshaw, L.B., M.D. Peyton, L.T. Archer, M.R. Black, J. Colsin, and L.S. Greenfield. Prevention of death in endotoxin shock by glucose administration. Surg. Gynecol. Obstet. 138:851-859, 1974.

3. Filkins, J.P., and R.P. Cornell. Depression of hepatic gluconeogenesis and the hypoglycemia of endotoxin shock. Am. J. Physiol. 227:778-781, 1974.

4. Berry, L.J., D.S. Smythe, and L.S. Colwell. Inhibition of hepatic enzyme induction as a sensitive assay for endotoxin. J. Bacteriol. 96:1191-1199, 1968.

5. Wilmore, D.W., C.W. Goodwin, L.M. Aulick, M.C. Powanda, A.D. Mason and B.A. Pruitt, Jr. Effect of injury and infection on visceral metabolism and circulation. Ann. Surg. 192:491-550, 1980.

6. Wolfe, R.R., D. Elahi and J.J. Spitzer. Glucose kinetics in dogs following a lethal dose of endotoxin. Metabolism 26:847-850, 1977.

7. Clowes, G.H.A., T.F. O'Donnell, N.T. Ryan and G.L. Blackburn. Energy metabolism in sepsis: Treatment based on different patterns of shock and high output stage. Ann. Surg. 179:684-696, 1974.

8. Neufeld, H.A., J.A. Pace and F.E. White. The effect of bacterial infections in ketone concentrations in rat liver and blood and on free fatty acid concentration in rat blood. Metabolism 25:877-884, 1976.

9. Fisher, R.H., J.C. Denniston and W.R. Beisel. Infection with diplococcus pneumonia and Salmonella typhimurium in monkeys: Changes in plasma lipoid and lipoproteins. J. Infect. Dis. 125:54-60, 1972.

10. Spitzer, J.J., A.A. Bechtel, L.T. Archer, M.R. Black and L.B. Hinshaw. Myocardial substrate utilization in dogs following endotoxin administration. Am. J. Physiol. 227:132-136, 1974.

13. Gallin, J.E., D. Kaye and W.M. O'Leary. Serum lipids during infection. N. Engl. J. Med. 281: 1081-1086, 1969.

14. Spitzer, J.A., A.G.B. Kovach, P. Sandor, J.J. Spitzer and R. Storck. Adipose tissue and lipolysis during endotoxin shock. Acta Physiol. Acad. Sci. Hung. 44:183-194, 1973.

15. Spitzer, J.A. Endotoxin-induced alterations in isolated fat cells: Effect on nor-epinephrine-stimulated lipolysis and cyclic 3', 5'-adenosine monophosphate accumulation. Proc. Soc. Exp. Biol. Med. 145:186-191, 1974.

16. Himms-Hagen, J. Adrenergic receptors for metabolic responses in adipose tissue. Fed. Proc. 29:1081-1086, 1969.

17. Carpentier, Y.A., J. Askanazi, D.H. Elwyn, M. Jeevanandam, F.E. Gump, A.I. Myman, J.M. Kinney and R. Burr. Effects of hypercaloric glucose infusion on lipid metabolism in injury and sepsis. J. Trauma. 19:649-654, 1979.

18. Grundy, S.M. Hypertriglyceridemia: mechanisms, clinical significance and treatment. Med. Clin. N. Am. 66:519-535, 1982.

19. Jeejeebhoy, K.N., B. Langer, G. Tsallas, R.C. Chu, A. Kuksis and G.H. Anderson. Total parenteral nutrition at home: studies in patients surviving 4 months to 5 years. Gastroenterology 71:943-953, 1976.

20. Krauss, R.M., H.G. Windmueller, R.I. Levy and D.S. Frederickson. Selective measurement of two different triglyceride lipase activities in rat postheparin plasma. J. Lipid Res. 14:286-295, 1973.

21. Haft, D.E. Effects of insulin on glucose metabolism by the perfused normal rat liver. Am. J. Physiol. 213:219-230, 1967.

22. Kissebah, A.H., S. Alfarsi, P.W. Adams and V. Wynn. Role of insulin resistance in adipose tissue and liver in pathogenesis of endogenous hypertriglyceridemia in man. Diabetologia 12:563-571, 1976.

23. Steiner, G., F.J. Haynes, G. Yoshimo, M. Vranic. Hyperinsulinemia and in vivo very-low-density lipoprotein-triglyceride kinetics. Am. J. Physiol. 246:E187-E192, 1984.

24. Tobey, T.A., M. Greenfield, F. Kraemer and G. M. Reaven. Relationship between insulin resistance, insulin secretion, very-low-density lipoprotein kinetics and plasma triglyceride levels in normotriglyceridemic man. Metabolism 30:165-171, 1980.

25. Hopkins, P.N., and R.R. Williams. A simplified approach to lipoprotein kinetics and factors affecting serum cholesterol and triglyceride concentrations. Am. J. Clin. Nutr. 34:2560-2590, 1981.

26. Romanosky, A.J., G.J. Bagby, E.L. Bockman and J.J. Spitzer. Free fatty utilization by skeletal muscle after endotoxin administration. Am. J. Physiol. 239:E391-E395, 1980.

27. Shaw, J.H.F. and R.R. Wolfe. Conscious dog model with hemodynamic and metabolic response similar to man. Surgery 95:553-561, 1984.

28. Wolfe, R.R. and J.H.F. Shaw. Inhibitory effect of plasma palmitate on glucose production in the conscious dog. Am. J. Physiol. 246:E181-E186, 1984.

29. Durkot, M.J. and R.R. Wolfe. Effects of adrenergic blockade on glucose kinetics in septic and burned guinea pigs. Am. J. Physiol. 241:R222-R227, 1981.

30. Wolfe, R.R., J.E. Evans, C.J. Mullany and J.F. Burke. Measurement of plasma fatty free acid turnover and oxidation using [1-$^{13}$C]-palmitic acid. Biomed. Mass. Spectrom. 7:168-171, 1980.

31. Lorenzi, M., J.J. Karam, E. Tsalikian, N.V. Bohannon, J.E. Gerich, P.H. Forsham. Dopamine during $\alpha$ and $\beta$-adrenergic blockade in man. J. Clin. Invest. 63:310-313, 1979.

32. Wolfe, R.R., M.J. Durkot and M.H. Wolfe. Investigation of kinetics of integrated metabolic response to adrenergic blockade in conscious dogs. Am. J. Physiol. 241:E385-E395, 1981.

33. Albano, J.D.M., R.P. Elkins, G. Mortiz and R.C. Turner. A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol. (Copehn). 70:487-509, 1972.

34. Faloona, G.R., and R.H. Unger. Glucagon. In: Methods of Hormone Radioimmunoassay, (eds)., G.M. Jaffe and H. R. Behrman. New York, Academic Press, 1974, pp. 317-330.

35. McDonald-Gibson, R.G. and M. Young. The use of automatic solids injection system for quantitative determination of plasma long chain non-esterified fatty acids by gas-lipid chromatography. Clin. Chem. Acta 53:117-126, 1974.

36. O'Keefe, S.J.D., P.M. Sender and W.P.T. James. Catabolic loss of body nitrogen in response to surgery. Lancet 2:1035-1038, 1974.

37. Steele, R. Influence of glucose loading and of injected insulin hepatic glucose output. Ann. N.Y. Acad. Sci. 82:420-430, 1959.

38. Wolfe, R.R. and J.J. Burke. Glucose and lactate metabolism in experimental septic shock. Am. J. Physiol. 235:R219-R227, 1978.

39. Issekutz, B., Jr., P. Paul, and H.I. Miller. Metabolism in normal and pancreatectomized dogs during steady-state exercise. Am. J. Physiol. 213:857-862, 1967.

40. Biesel, W.R. A role for glucagon during infection. N. Engl. J. Med. 288:734-735, 1973.

41. Sacca, L.R., R. Sherwin and P. Felig. Influence of somatostatin on glucagon and epinephrine stimulated hepatic glucose output in the dog. Am. J. Physiol. 236:E113-E117, 1979.

42. Zenser, T.V., F.R. DeRubertis, D.T. George and E.F. Rayfield. Infection-induced hyperglucagonemia and altered hepatic response to glucagon in the rat. Am. J. Physiol. 227:1299-1305, 1974.

43. Cherrington, A.D., J.L. Chiasson, J.E. Liljenquist, A.S. Jennings, U. Keller and W.W. Lacy. The role of insulin and glucagon in the regulation of basal glucose production in the postabsorptive dog. J. Clin. Invest. 58:1407-1418, 1976.

44. Wolfe, R.R. and M.J. Durkot. Evaluation of the role of the sympathetic nervous system in the response of substrate kinetics and oxidation to burn injury. Circ. Shock 9:395-406, 1982.

45. Rizza, R.A., J.E. Gerich. Persistant effect of sustained hyperglucagonemia on glucose production in man. J. Clin. Endo. Metab. 48:352-355, 1979.

46. Cahill, S.F. Starvation in man. N. Engl. J. Med. 282:668-675, 1970.

47. Wolfe, R.R. and J.H.F. Shaw. Inhibitory effect of plasma palmitate on glucose production in the conscious dog. Am. J. Physiol. 246:E181-E186, 1984.

48. Wilmore, D.W., J.M. Long, A.D. Mason, Jr., R.W. Skrean and B.A. Pruitt, Jr. Catecholamines: Mediators of the hypermetabolic response to thermal injury. Ann. Surg. 180:653-669, 1974.

49. Gallin, J.I., D. Kaye and W.M. O'Leary. Serum lipids in infection. N. Engl. J. Med. 281:1081-1086, 1982.

50. Griffiths, J., A.C. Groves and F.Y.T. Leung. The relationship of plasma catecholamines to serum triglycerides in canine gram-negative bacteremia. Surg. Gynec. Obstet. 134:795-802, 1972.

51. Kauffmann, R.L., C.F. Matson and Biesel, W.R. Hypertriglyceridemia produced by endotoxin: role of impaired triglyceride disposal mechanisms. J. Infect. Dis. 133:548-555, 1976.

52. Wolfe, R.R., and G.J. Bagby. Lipid metabolism in shock. In Handbook of Shock and Trauma, Altura, B.M., A. Lefer and W. Shumer (eds)., Raven Press, New York, 1983, pp. 199-216.

52-a. Verschoor, L., and Y.D.I. Chen, G.M. Reaven. In search of a relationship between physiologically-induced variations in adipose tissue lipoprotein lipase activity and very low density lipoprotein kinetics in normal rats. Metabolism. 31:499-503, 1982.

53. Bagby, G.J., J.A. Spitzer, H.I. Miller and J.J. Spitzer. Increased tissue lipoprotein lipase activity in guinea pigs following burn trauma. Circ. Shock 8:131-136, 1981.

54. Bagby, G.J., and J.A. Spitzer. Lipoprotein lipase in rats sensitized to endotoxin by surgical trauma. J. Surg. Res. 29:110-115, 1980.

55. Bagby, G.J., and J.A. Spitzer. Lipoprotein lipase activity in rat heart and adipose tissue during endotoxic shock. Am. J. Physiol. 238:H325-H330, 1980.

56. Border, J.R., G.P. Burns, C. Rumph and W.G. Schenk, Jr. Carnitine levels in severe infection and starvation: a possible key to the prolonged catabolic state. Surgery 68:175-179, 1970.

57. Torosian, M.E., R. Fried, G.P. Buzby and T.P. Stein. The effect of caloric source on lipid and protein metabolism in septic rats (abstract). J.P.E.N. 8:86, 1984.

58. Wolfe, R.R., and M.J. Durkot. Evaluation of the role of the sympathetic nervous system in the response of substrate kinetics and oxidation to burn injury. Circ. Shock 9:395-406, 1982.

59. Brindley, D.N. Regulation of hepatic triacylglycerol synthesis and lipoprotein metabolism by glucocortiacords. Clin. Sci. 61:129-133, 1981.

60. Cherrington, A.D., J.L. Chiasson, J.E. Liljenquist, A.S. Jennings, U. Keller and W.W. Lacy. The role of insulin and glucagon in the regulation of basal glucose production in the post-absorptive dog. J. Clin. Invest. 58:1407-1418, 1976.

61. Cherrington, A.D., W.W. Lacy, P.E. Williams and K.E. Steiner. Failure of somatostatin to modify effect of glucagon on carbohydrate metabolism in the dog. Am. J. Physiol. 244:E596-E602, 1983.

62. Wolfe, R.R., Shaw, J.H.F. Inhibitory effect of plasma FFA on glucose production in the conscious dog. Am. J. Physiol. 246:E181-E186, 1984.

63. Ginsberg, H.N., A. Jacobs, N.A. Le and J. Sandler. Effect of somatostatin-induced suppression of post-prandial insulin response upon the hypertriglyceridemia associated with a high carbohydrate diet. J. Clin. Invest. 70:1225-1233, 1982.

64. Shulman, G.I., P.E. Williams, J.E. Liljenquist, W.W. Lacy, U. Keller and A.D. Cherrington. Effect of hyperglycemia, independent of changes in insulin and glucagon on lipolysis in the conscious dog. Metabolism 29:317-320, 1980.

65. Barter, P.J., P.J. Nestel and K.F. Carroll. Precursors of plasma triglyceride fatty acid in humans: effects of glucose consumption, clofibrate administration and alcoholic fatty liver. Metabolism 21:117-124, 1972.

66. Wolfe, R.R., T.F. O'Donnell, M.D. Stone, D.A. Richmand and J.F. Burke. Investigation of factors determining the optimal glucose infusion rate in total parenteral nutrition. Metabolism 29:892-900, 1980.

67. Mullany, C.J., R.R. Wolfe and J.F. Burke. The rate of glucose infusion in fasting and fed guinea pigs: Glucose oxidation rates and the distribution of glucose in liver, muscle and adipose tissue. J. Surg. Res. 29:116-125, 1980.

68. Letarte, J. and T.R. Fraser. Stimulation by insulin of the incorporation of $U^{14}C$-glucose into lipids released by the liver. Diabetologia 5:358-359, 1969.

69. Dich, J., B. Bro, N. Grunnet, F. Jensen and J. Kondrup. Accumulation of triacylglycerol in cultered rat hepatocytes is increased by ethanol and by insulin and dexamethasone. Biochem. J. 212:617-623, 1983.

## FIGURE LEGENDS

Figure 1. Results from column chromatography (Sephadex LG-20) showing that triglyceride from labeled VLDL eluted in a single peak that was distinct resulting from the addition of 1-$^{14}$C-palmitate.

Figure 2. Glucose metabolism in control and septic dogs and the effect of hormonal control and sympathetic blockade. Hormonal control was induced by the infusion of somatostatin (0.5 μg/kg·min), insulin (0.2 mU/kg·min) and glucagon (0.9 ng/kg·min). Sympathetic blockade was induced by the infusion of propranolol (2.0 mg/kg) and phentolamine (1.5mg/kg). Period 1 lasted 90 min; Period 2 was 60 min; and Period 3 was 60 min (n=7).
*Significantly different from preceeding period in the same group ($P<0.05$).
+Significantly different from control value in the corresponding period ($P<0.05$).

Figure 3. The effects on palmitate metabolism in control and septic dogs, of hormonal control and sympathetic blockade. Infusions and sampling as in Figure 1 (n=7).
*Significantly different from the preceeding period in that group ($P<0.05$).
+Significantly different from the control value for that period ($P<0.05$).

Figure 4. Plasma glucose and insulin concentrations during period 3 in both protocols. The value at 0 min refers to the average value during period 2 (hormonal control alone) for each group. In all groups, somatostatin, insulin and glucagon were infused throughout periods 2 and 3. Hyperinsulinemia was produced by increasing the insulin infusion rate from 0.2 mU/kg·min to 0.8 mU/kg·min, and hyperglyceria was produced by infusing glucose at the rate of 7 mg/kg·min, without altering the insulin infusion rate. During the hyperinsulinemia, glucose was infused at a variable rate (approximately 4 mg/kg·min) in order to maintain euglycemia.
*Significantly different from period 2 value for that group ($P<.05$).

Figure 5. Effect of hyperglycemia or hyperinsulinemia on the rate of release into plasma of VLDL (RaVLDL) and FAA (RaFAA). The hormonal control value represents the pooled values from both protocols in period 2, since there were no significant differences. For statistical purposes, the value in period 3 were compared with the value from period 2 for the same group of dogs only. Units for RaVLDL are μmole TG/kg·min.
*Significantly different from period 2 value for that group ($P<.05$).

FIGURE 1

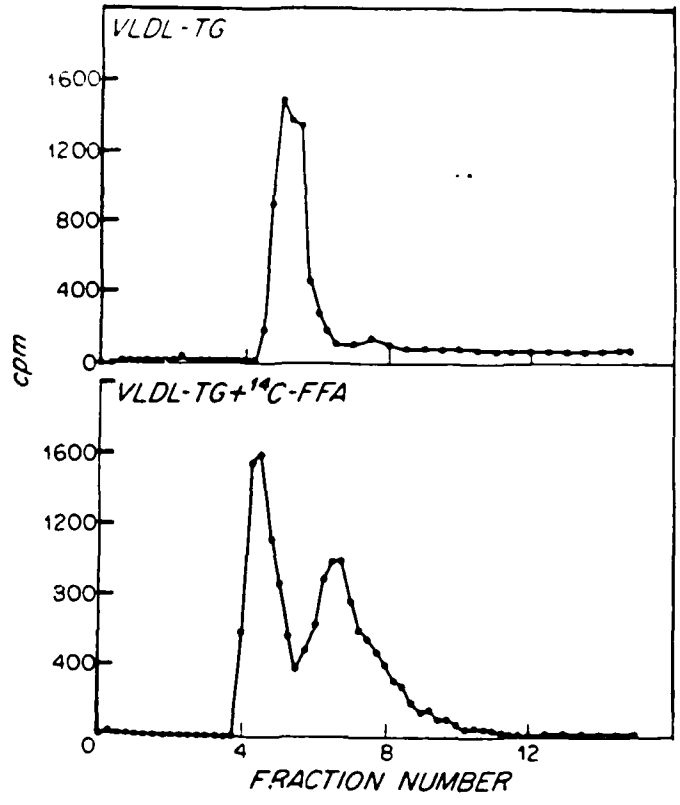
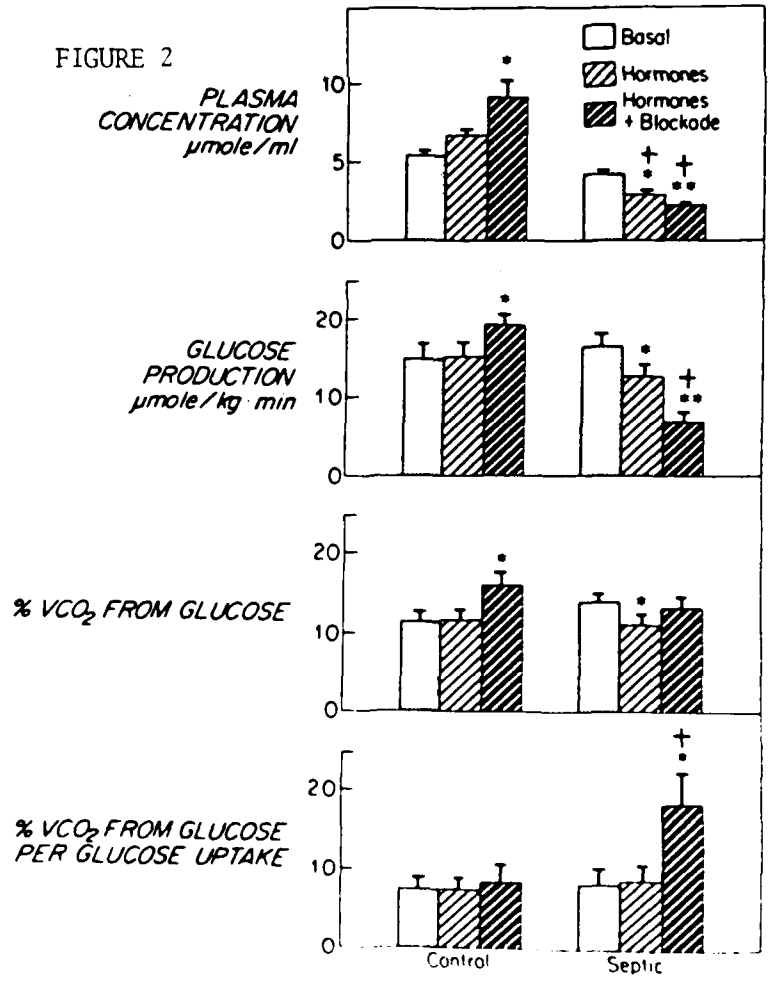



FIGURE 2



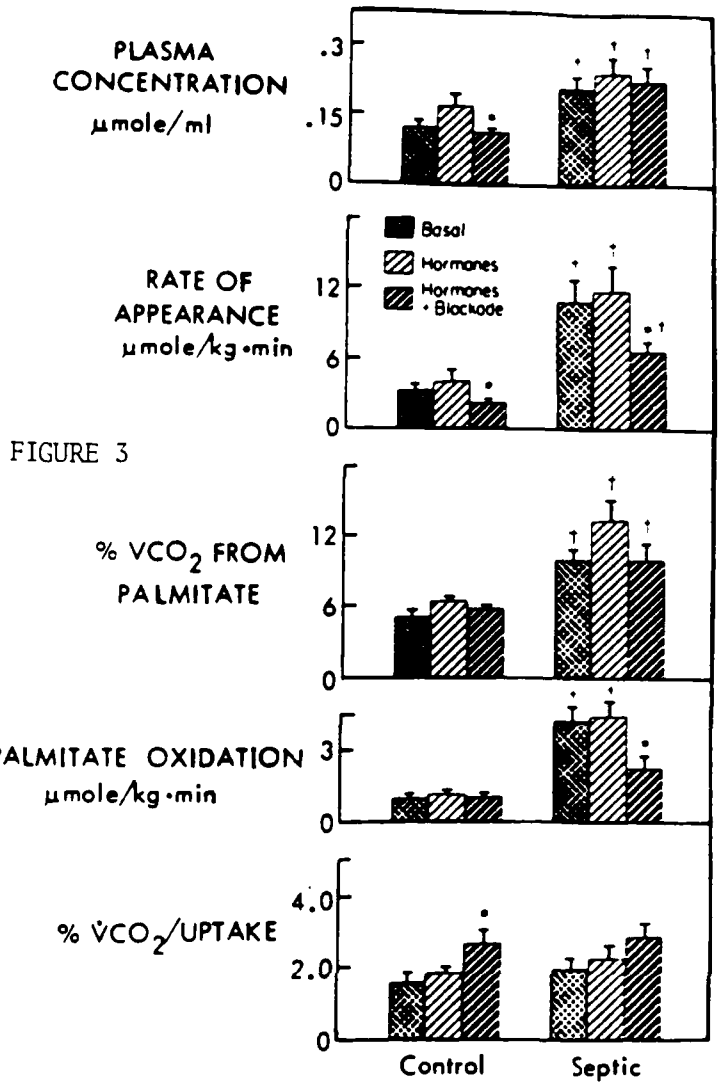



FIGURE 3

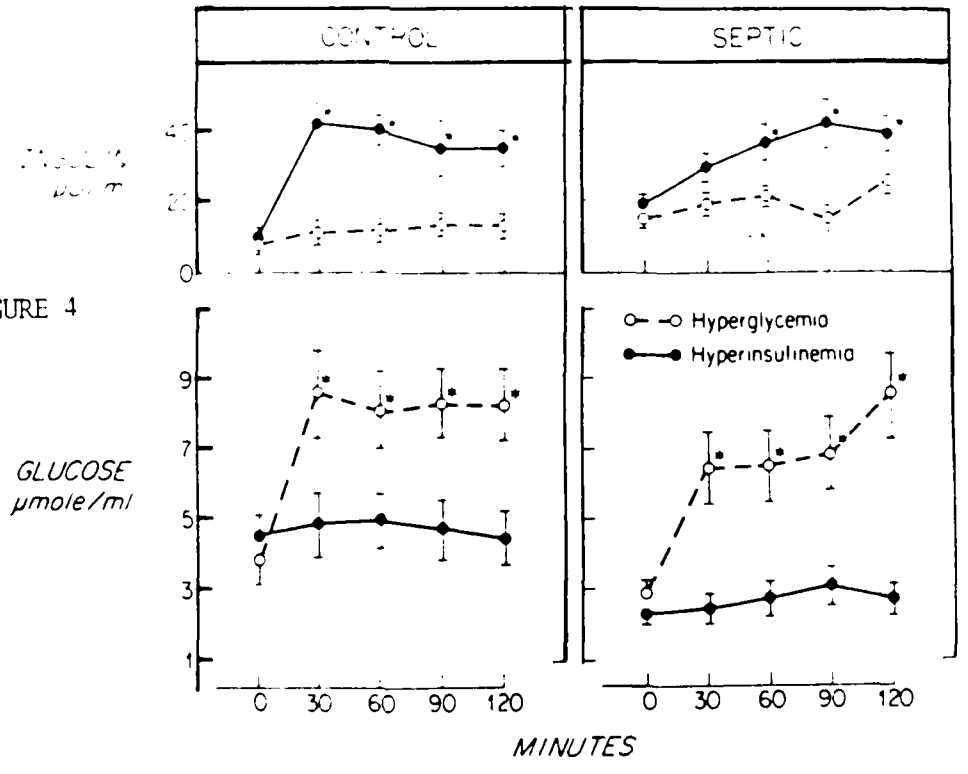



FIGURE 4

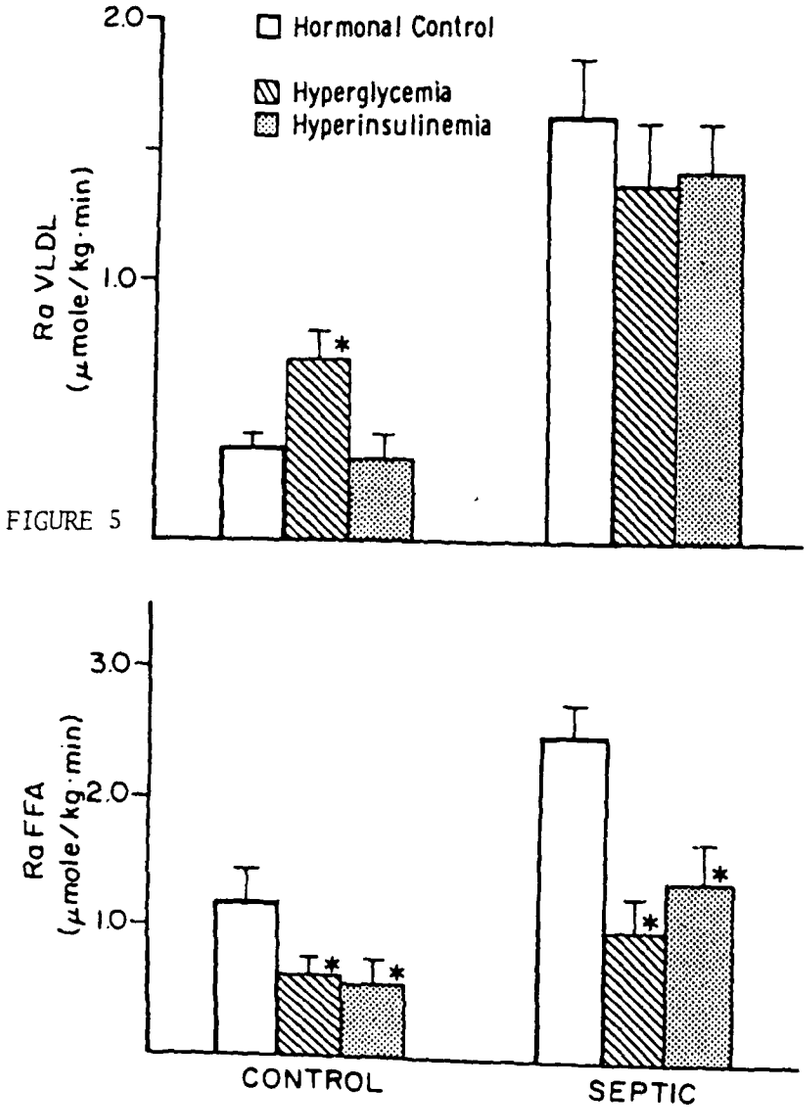

Hormonal Control
Hyperglycemia
Hyperinsulinemia
Ra VLDL
(μmole/kg·min)
2.0
1.0
*
Ra FFA
(μmole/kg·min)
3.0
2.0
1.0
*
*
*
*
CONTROL
SEPTIC

FIGURE 5

TABLE 1

PLASMA CONCENTRATIONS OF INSULIN AND GLUCAGON

| | INSULIN (μU/mL) | GLUCAGON (pg/mL) |
|---|---|---|
| Control Animals (n = 7) | | |
| Basal | 7.8 ± 1.1 | 227 ± 71 |
| SIG[1] | 11.7 ± 1.3 | 204 ± 15.6 |
| SIG + Blockade | 13.4 ± 3.8 | 200 ± 19 |
| Septic Animals (n = 7) | | |
| Basal | 20.4 ± 3.1[3] | 912 ± 50[3] |
| SIG | 20.4 ± 5.6[3] | 281 ± 41[2,3] |
| SIG + Blockade | 17.6 ± 4.2 | 196 ± 30 |

1. Infusion of somatostatin (0.5 μg/kg·min), insulin (0.2 mU/kg·min) and glucagon (0.9 ng/kg·min) in controls and 0.5 μg/kg·min, 0.2 mU/kg·min, and 1.8 ng/kg·min, respectively in septic animals.
2. Significantly different from basal value for same group ($P < 0.05$).
3. Significantly different from control value for corresponding period ($P < 0.05$).

TABLE 2

EFFECT OF SEPSIS ON BASAL KINETICS

| | Triglyceride Concentration (umole/mL) | RaVLDL (umole/kg·min) | FFA Concentration (umole/mL) | RaFFA (umole/kg·min) |
|---|---|---|---|---|
| Controls | 0.53 ± 0.07 | 0.29 ± 0.05 | 0.42 ± 0.05 | 12.5 ± 1.37 |
| Dogs with Sepsis | 0.92 ± 0.17* | 1.63 ± 0.24* | 0.70 ± 0.07* | 23.8 ± 2.9* |

Values are $\bar{X}$ ± SEM.

*Significantly different from control value ($P < .01$).

TABLE 3

RATIO OF RaVLDL/RaFFA IN CONTROL AND SEPTIC DOGS

| | Basal | Somatostatin + Hormonal Control (Period 2) | Hyperglycemia (Period 3) | Hyperinsulinemia (Period 3) |
|---|---|---|---|---|
| Controls | 0.024 ± 0.009 | 0.030 ± 0.012 | 0.124 ± 0.040* | 0.077 ± 0.027* |
| Dogs with sepsis | 0.069 ± 0.033+ | 0.093 ± 0.028+ | 0.136 ± 0.031 | 0.102 ± 0.040 |

Values are $\bar{X}$ ± SEM.

*Significantly different from corresponding period 2 value.

+Significantly different from control value for same period ($P < .05$).

TABLE 4

RATIO OF RaVLDL/RaFFA IN CONTROL AND SEPTIC DOGS

| | Basal | Somatostatin + Hormonal Control (Period 2) | Hyperglycemia (Period 3) | Hyperinsulinemia (Period 3) |
|---|---|---|---|---|
| Controls | 0.024 ± 0.009 | 0.030 ± 0.012 | 0.124 ± 0.040* | 0.077 ± 0.027* |
| Dogs with sepsis | 0.069 ± 0.033+ | 0.093 ± 0.028+ | 0.136 ± 0.031 | 0.102 ± 0.040 |

Values are $\bar{X}$ ± SEM.

*Significantly different from corresponding period 2 value.

+Significantly different from control value for same period ($P < .05$).

END

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